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MYELIN STRUCTURES IN LIPOIDS AND THEIR RELATION TO ELECTRIC CHARGES.

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In a paper by Dr. G. W. Crile (1), reference is made to the myelin structures which grow out from lipoid films covered with various solutions. It was observed that the application of electrical polarization increased the growth of these myelin structures. In this paper further studies are published with the aim of correlating the growth of myelin structures with other physical properties.

The formation of myelin structures was first described by Virchow (2) in 1854. Haeckel (3) assigned an important role to these structures in vital phenomena; Lehmann (4) and Rhumbler (5) made further experiments. Recently Leathes (6) investigated the myelin structures formed by various lipoids. He took moving pictures of their growth and influenced the rate of growth by applying various salt solutions in different concentrations. It appeared that concentrated salt solutions retarded growth, while more dilute solutions stimulated it. Calcium salts inhibited growth. Various types of structures were obtained in solutions containing gelatin, egg white, serum hemoglobin, etc., and with mixtures of lecithin and cholesterol.

In our own investigations an attempt was made to bring this phenomenon into relationship with the electrical surface charge of thin films of lipoids. The basis for expecting that such a relationship is possible is the known fact that in thin lipoid films an orientation of the fatty acid molecules takes place (Leathes (7) and Adam (8)). The fatty acid group is attracted toward the water, while the methyl group of the paraffin chain is directed away from it. The fatty acid chains are arranged

parallel to each other, each lecithin molecule for instance occupying a surface of 56 square Å, according to Leathes' measurements on thin films spread over water. Trillat (9) found a similar parallel arrangement of fatty acid molecules with the Hull-Debye-Scherrer x-ray method. On films spread over metals he found that the distance between the lecithin molecules averaged 47 Å, which is a somewhat higher value than for films spread over water. Further indications of the polar arrangement of lipoid molecules are shown by the model experiments of Runnstorm (10) in which the molecules show double refraction and striated lamellae under the polarization microscope.

Actual measurements of the surface charge of cholesterol sols were made by Remesow (11) by the cataphoresis method, which gave interesting results; this method, however, could not be applied to lecithin emulsions.

In seeking a possible explanation for the formation of myelin structures the following observation is of value. The myelin structure is always surrounded by a thin film (Fig. 2B) while the inside of the structure is filled with liquid. In a dry particle of lipoid the fatty acid chains are necessarily orientated at random. If this particle is then surrounded with pure water, an immediate rearrangement of the fatty acid chain takes place in such a way that the acid ends point toward the water, while the paraffin ends point toward the inside of the particle. Water passes through between the orientated fatty acid chains, and consequently the surface of the lipoid particle begins to enlarge, long myelin structures rising out of the lipoid. The growth velocity of the myelin fibers must necessarily depend on the velocity with which the water penetrates through the surface film and increases it. This phenomenon might be explained as being due to various physical causes, like diffusion, swelling (similar to that of proteins), osmosis, surface tension, etc. This will be discussed later.

EXPERIMENTAL METHOD.

To avoid the difficulty of working with lecithin an ether extract of calf's brain was used. The tissue was thoroughly minced, rapidly dried at a temperature not exceeding 40° C. (higher temperatures lessen or inhibit the growth of myelin structures). The dried tissue was pulverized, extracted with ether, and filtered several times. This solution can be kept

for several weeks. Extracts which were over a month old were not used. The concentration of the ether solution was determined by evaporating a known amount of the solution to dryness under vacuum. Generally the ether solution was so diluted as to contain one per cent lipoid.

The growth of the myelin structures was observed in the following way. A well slide (similar to those used in tissue cultures) was thoroughly cleaned and a drop of the ether solution was deposited on it by means of a glass rod. The drop was of such dimensions that it corresponded to 0.1 cc. of the one per cent lipoid solution, containing therefore about 1 mg. of lipoid. The drop was deposited so that it formed a round, thin film about 5–7 mm. in diameter. The ether evaporated very rapidly, leaving a very thin film, which was generally somewhat thicker around the edge. This film was covered with several drops of the solution, the effect of which was to be tested, and a cover glass was placed over it. Slight pressure on the cover glass expelled the excess of liquid, which was blotted off, leaving a preparation which without drying could be observed over a period of several days. Vaseline or other sealing material was found to be unnecessary.

On account of the thinness of the film, the growth of myelin structures could be observed not only on the edge of the film, but throughout its surface. The standard comparison of growth was made with the help of a solution which contained the salts found in the brain dissolved in distilled water in the same proportion as they are present in the brain. The composition of 1000 cc. of this "brain-salt" solution was as follows: KCl—2.33 gm., Na_3PO_4—2.21 gm., K_3PO_4—0.89 gm., Na_2CO_3—0.11 gm., K_2SO_4—0.24 gm., $CaCl_2$—0.026 gm., $MgCl_2$—0.55 gm., distilled water to make 1000 cc. The calcium and magnesium salts formed a precipitate with the phosphate, which was filtered off. This solution was alkaline—pH 11.4. Myelin structures were readily formed in this solution. Immediately after a lipoid film was covered with this solution, small myelin growths appeared, not only on the edge of the film, but all over its surface. With a magnification of 600 the growth of the structures could be observed and with the aid of an ocular micrometer the growth could be readily followed.

In order to compare the effects of solutions of various salts in distilled water, a number of well slides were covered with the thin lipoid film; the various solutions were superimposed

as quickly as possible and the growth of the myelin structures was observed under the microscope at intervals of 5, 10 and 30 minutes. The results are given in Table I in which the best growths (that is, one comparable to that in the brain-salt solution) are indicated by xxx, medium growths by xx, slight growths by x and no growth at all by —.

TABLE I.

GROWTH OF MYELIN STRUCTURES IN VARIOUS SOLUTIONS.

SOLUTION	CONCENTRATION OF SOLUTION IN MOLS.					
	1	0.1	0.01	0.001	0.0001	0.00001
NaCl	—	x	xx	xxx	xxx	xx
KCl	—	x	xx	xx	xx	xx
$CaCl_2$	—	—	—	x	xx	xx
$MgCl_2$	—	—	—	x	xx	xx
$CuCl_2$	—	—	—	x	xx	xx
$FeCl_3$	—	—	—	—	x	xx
HCl	—	—	—	—	x	x
NaOH	x	xx	xx	xxx	xx	xx
K_3PO_4	x	xx	xx	xxx	xxx	xx
K_2SO_4	x	xx	xxx	xx	x	x
KCN	x	xx	xxx	xx	x	x
NaI	x	x	xx	x	x	x
Na_2CO_3	x	xx	xxx	xx	x	x

In solutions of the chlorides the growth increased in direct relation to the degree of dilution. The bivalent salts inhibited growth in 0.01 molar solutions and the trivalent $FeCl_3$ inhibited growth even in 0.001 molar solutions. No growth was observed in acid solutions except in those in which the pH was above 3.6, while NaOH and K_3PO_4 favored growth even in higher concentrations. Anions such as SO_4, CN, CO_2 seemed to favor growth as compared with Cl.

The characteristic growths produced by different concentrations are indicated in Fig. 1, which shows the effect of (A) n, (B) 0.01n and (C) 0.001n NaCl solutions on the growth of myelin structures; while Table I gives the approximate grades of growth (—, x, xx, and xxx, respectively). Non-electrolytes have a marked influence on growth. For instance, an xx growth appears in a 10 per cent solution, glucose and even a saturated solution of glucose is not able to stop growth. Various dilutions of ethyl alcohol also exhibit a marked influence.

FIG. 1. Photomicrographs showing the effect of NaCl solutions on the growth of myelin structures.

A: n NaCl, growth—cataphoretical charge, 0 millivolt.
B: 0.1 n NaCl, xx, cataphoretical charge, —51.
C: 0.001 n NaCl, xxx, cataphoretical charge, —87.

FIGURE 1.

In mixtures of salt solutions an antagonistic effect can be observed. If a growth-inhibiting solution of $CaCl_2$ is mixed with a growth-favoring solution, a fair growth of structures can be seen. In order to compare the antagonistic effects of various solutions, a further series of observations was made by dissolving the various agents in the "brain-salt" solution instead of in distilled water (Table II). In this way the action

TABLE II.

GROWTH OF MYELIN STRUCTURES IN A MIXTURE OF VARIOUS SOLUTIONS WITH THE BRAIN SALT SOLUTION.

SOLUTION	CONCENTRATION OF SOLUTION IN MOLS.				
	1	0.1	0.01	0.001	0.0001
NaCl	x	xx	xxx	xxx	xxx
KCl	x	x	xx	xxx	xxx
$CaCl_2$	—	—	x	xx	xxx
$MgCl_2$	—	—	x	xx	xxx
$FeCl_3$	—	—	—	xxx	xxx
$AlCl_3$	—	—	—	xxx	xxx
NaI	xx	xx	xx	xxx	xxx
NaBr	xx	xxx	xxx	xx	xxx
	CONCENTRATION OF SOLUTION IN PER CENTS				
	1	0.1	0.01	0.001	0.0001
Adrenalin	.....	x	xxx	xx	xxx
Atropin	x	xx	xxx	.....	.....
Caffein	xxx	xxx	xxx	.....	.....
Luminal	xx	xx	xxx	.....	.....
Strychnin	x	xx	xx	xxx	.....
Ethyl alcohol	x 50%	xxx 25%	xx 10%	xxx 2.5%	.....
Formaldehyd	— 40%	x 10%	xx 1%	xxx 0.1%	.....
Glucose	x 20%	xx 10%	xxx 1%	.....	.....

of the agents is observed in the same salt solution as that which is present in the brain, although it is in no way implied that in this way a comparison between the effect of agents on the lipoid emulsion and on the brain could be made directly.

Some of the metal chlorides form a precipitate with the phosphate, etc., which results in the removal of the added metal ions from the solution. As shown in Table II, however, the salts exert a marked influence although their inhibiting action

in the higher concentrations is counterbalanced by the "brain-salt" solution.

The effect of alcohol is shown in Fig. 2. The inhibiting effect of a 50 per cent solution (C), and the accelerating effect of a 25 per cent solution (B) is evident. The inhibiting effects of some of the other agents in relatively low concentrations are remarkable.

CATAPHORESIS MEASUREMENTS.

A slightly modified Northrop cataphoresis apparatus was used. The current was conducted through reversible electrodes. The charge of the particles was calculated from the Helmholtz-Schmoluchowski formula. With concentrated solutions the viscosity of the solution was also taken into account.

The emulsion was obtained by drying some lipoid thoroughly and mixing it with the solution to be tested. After mixing, the usual myelin formations grew out of the mass of lipoid and when the mixture was shaken for some time the longitudinal parts of the long myelin structures could be seen floating in the mixture. When the mixture was shaken vigorously, the myelin structures were broken up into oblong tubular particles which were filled with liquid. The length of these particles was generally about $20\mu\mu$, although some larger particles were found occasionally. When the suspension was allowed to stand for several hours practically the whole of the lipoid was changed into such particles.

Another method for preparing emulsions was to mix the ether solution of the lipoid with distilled water or the salt solution to be tested. As much of the ether as possible was evaporated under reduced pressure at a temperature of 40° C. In this way a suspension of round globules was obtained, in which a rapid growth of myelin structures could be seen.

The emulsion of myelin structures was placed in the cataphoresis apparatus and the velocity of the particles in an electric field was measured with a stop watch. The necessary precautions in regard to differences in the charge in the capillary tube were taken by always measuring the velocity at the same level of the capillary; in this way all the observations could be compared. While there might be some theoretical objection to the use of the formula, as the charge was always calculated from the same formula only relative values were compared. Some authors prefer to give the cataphoretical velocities as measured directly.

FIGURE 2.

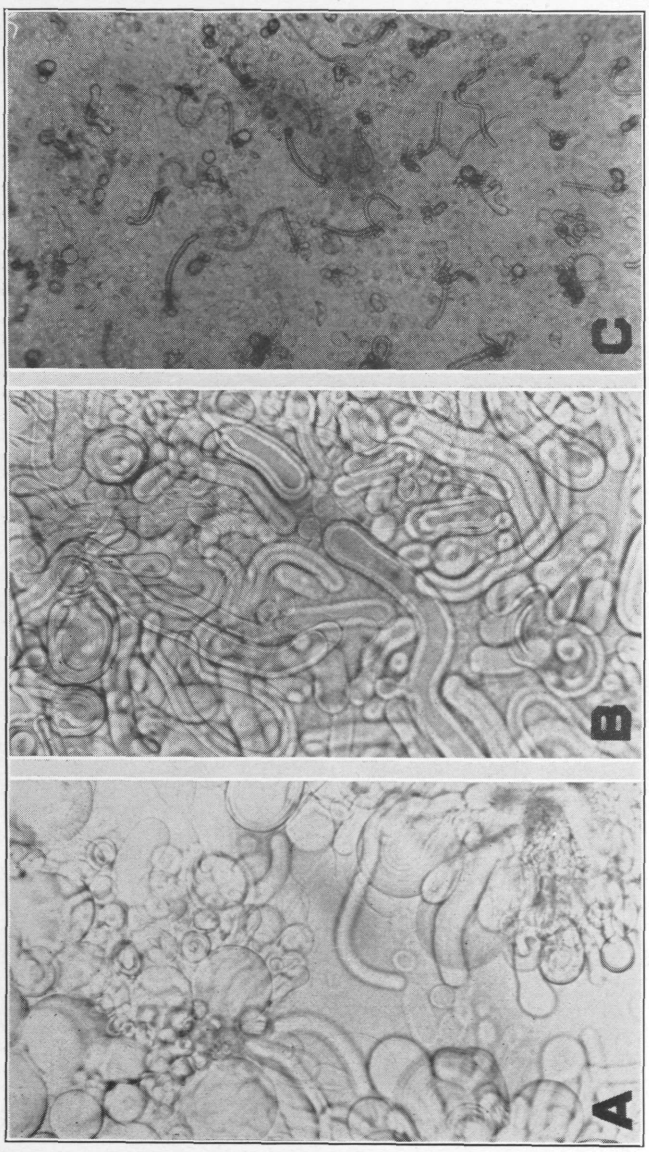

FIG. 2. Photomicrographs showing the effect of ethyl alcohol solution on the growth of myelin structures.
A: 10 per cent solution growth xxx, cataphoretical charge, —62 millivolts.
B: 25 per cent solution xxx, cataphoretical charge, —75.
C: 50 per cent solution x, cataphoretical charge, —18.

In Table III are given the charges in millivolts of myelin particles in the various solutions. The charge of the lipoid in distilled water alone is —78 millivolts. Very dilute salt solutions increase the charge, while concentrated salt solutions generally decrease the charge until the particles become discharged or their charge is even reversed. The best method of observation was to prepare a suspension in distilled water.

TABLE III.

GROWTH OF MYELIN STRUCTURES IN VARIOUS SOLUTIONS COMPARED WITH THEIR ELECTRIC CHARGE (MILLIVOLTS).

SOLUTION	CONCENTRATION OF SOLUTIONS IN MOLS.							
	1	0.5	0.1	0.01	0.001	0.0001	0.00001	H_2O
NaCl	—	—	x	xx	xxx	xxx	xx	.....
	0	—11	—51	—85	—87	.....	.....	.....
KCl	—	—	x	xx	xx	xx	xx	.....
	5	0	—43	—63	—73	.....	.....	.....
$CaCl_2$	—	—	—	—	x	xx	xx	.....
	7	0	0	—17	—49	.....	.....	.....
$CuCl_2$	—	—	—	—	x	xx	xx	xx
	.....	.....	.....	0	—46	—61	—70	—68
$FeCl_3$	—	—	—	—	—	x	xx	xx
	.....	0	38	66	0	—49	—51	—70
NaOH	x	xx	xx	xx	xxx	xx	xx	xx
	—12	.....	—48	—91	—108	—84	—89	—98
K_3PO_4	x	xx	xx	xx	xxx	xxx	xx	xx
	—15	.....	—36	—62	—111	—94	—78	—100
Ethyl alcohol	50%	25%	10%	1%	.....	.....	.....	.....
	x	xxx	xxx	xx	.....	.....	.....	.....
	—18	—75	—62	—43	.....	.....	.....	.....
Urethane	1%	0.1%	0.01%	0.001%	.....	.....	.....	.....
	—	x	x	—	.....	.....	.....	.....
	—17	—36	—47	—18	.....	.....	.....	.....

The cataphoresis cell was filled with the emulsion and the velocity of the particles determined; a sufficient amount of salt solution was then added, the emulsion was mixed in an attachment of the cataphoresis cell and the velocity determined again without removing the emulsion from the apparatus. In this way, successive concentrations of the same salt were determined for their effect in changing the electrical charge.

In distilled water the whole amount of the lipoid changes very quickly into myelin forms. If subsequently an agent is added which discharges the particles or reverses their charge, the small structures shrivel up or change into shapeless masses, the velocity of which may still be determined. On the other

hand, if the discharging agent is added directly to a compact mass of lipoid, no myelin formation occurs generally, and so it is impossible to measure the change of the charge.

The peculiar changes observed are similar to those found by Heesch (12), by Remesow (11) in observations on cholesterol particles and by Brown and Broom (13) in observations on bacteria. Nevertheless such changes are not characteristic of lipoid alone, as various other suspensions show similar changes in the charge during the influence of bivalent and trivalent salts. Obviously, a surface charge or a potential difference will not produce myelin structures in most substances, but naturally the conclusion will be drawn from Table III that *the growth of the myelin structures is proportional to their negative electrical charge,* in the solutions which were used in our experiments.

INFLUENCE OF GALVANIC CURRENT ON THE GROWTH OF MYELIN STRUCTURES ON A LIPOID FILM.

If the growth of myelin structures in various salt solutions is proportional to the electric charge or electric potential difference between the film and the salt solution, then it should be possible to increase this potential difference by applying a constant galvanic current through the film. In such a case an electric polarization will result on the film, which will act as an increased charge. The following experiment was conducted. A microscopic slide was equipped with two reversible electrodes (silver needles covered electrolytically with silver chloride) which were separated by an interval of about 1 cm. A small drop of lipoid was placed between the electrodes, and a few drops of "brain-salt" solution were superimposed, so that the solution covered the electrodes. An electric current of about 1.5 volts was then passed through the solution. The myelin structures immediately began to grow rapidly all over the drop of lipoid bending towards the positive pole of the applied current. The growth was compared with that observed in an entirely identical drop in an experiment which was made under the same conditions and at the same time, except that in the latter experiment the electrodes were omitted. B and C in Fig. 3 show the growth with and A without electrical polarization. It will be noted that the structures point directly towards the positive pole. These experiments show not only that the growth is proportional to the negative charge of the

FIGURE 3.

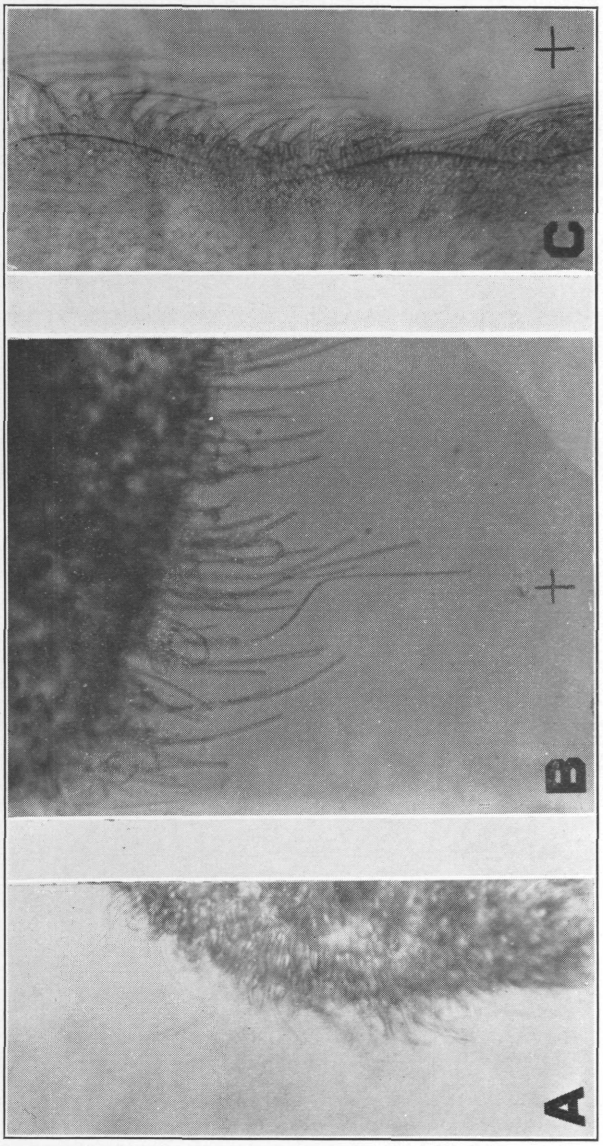

FIG. 3. Photomicrograph showing the effect of electrical polarization on the growth of myelin structures.
A: without polarization. B and C: with polarization.
Note the direction and bending (in C) of the myelin structures toward the positive pole of the applied current.

myelin structures in the various salt solutions, but also that in the same salt solution an artificially increased charge will produce a very much increased growth.

MEASUREMENTS OF SURFACE TENSION AND VISCOSITY.

The surface tension of the lipoid suspension was measured with Traube's stalagmometer and parallel measurements of viscosity were made with a capillary viscosimeter. The procedure was as follows: The viscosimeter was filled with the lipoid suspension (1 per cent lipoid in distilled water). The determinations were made, and the same sample was then transferred to the stalagmometer and surface tension readings were taken. The solution, the effect of which was to be tested, was then added to the suspension and the surface tension and viscosity determined again on the same sample.

TABLE IV.

GROWTH OF MYELIN STRUCTURES COMPARED WITH THE ELECTRIC CHARGE OF THE LIPOID AND THE SURFACE TENSION WITH AND VISCOSITY OF THE LIPOID EMULSION.

MATERIAL	RELATIVE SURFACE TENSION	RELATIVE VISCOSITY	ELECTRIC CHARGE MILLIVOLT	GROWTH
Lipoid suspension in distilled water.....	0.76	1.00	—98	xx
Same in 0.0001 n NaOH.....	0.89	1.08	—84	xx
Same in 0.001 n NaOH.....	0.93	1.00	—108	xxx
Same in 0.01 n NaOH.....	0.88	0.95	—91	xx
Same in 0.1 n NaOH.....	0.83	0.92	—48	xx
Same in 0.2 n NaOH.....	0.92	0.95	—40	xx

The relative values of the viscosity, surface tension, the electric charge in millivolts and the observed growth of myelin structures are compiled in Table IV. Values are given only for a lipoid suspension in NaOH solutions, as this is sufficient to illustrate that there is no relation between the surface tension or the viscosity and the growth of myelin structures. Other measurements were made with electrolytes and non-electrolytes.

DISCUSSION.

As was mentioned above, the basic explanation for the growth of myelin structures is that water actually penetrates through the lipoid-water interface. Such a penetration might be explained possibly by the presence of various physical changes like osmosis, diffusion, surface tension, etc. If this is true,

then the changes of the physical constants must result in a considerable difference between the effects of different solutions, as for example of NaCl and HCl, and furthermore the constants must change considerably with the dilution, as for example in n and in n/100 NaCl. It is evident that there is no proportionality between the physical properties of the solution and the growth of myelin structures.

SUMMARY.

1. The growth of myelin structures in lipoid extracts of mammalian brain was found to depend on the concentration of the solution. No myelin growth took place in concentrated solutions; with increasing dilution the growth reached a maximum and slightly decreased again with greater dilution.

2. Bivalent salts have a considerable inhibiting action even in n/100 molecular solutions, while trivalent salts inhibit growth even more.

3. An antagonistic action was found to take place in mixtures of salt solutions.

4. The growth of myelin structures is proportional to their negative electrical charge.

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